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IN VITRO ORGANOGENESIS OF *PELECYPHORA ASELLIFORMIS* ERHENBERG (CACTACEAE)

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Summary

In vitro propagation of *Pelecyphora aselliformis*, a Mexican cactus which is considered rare and is highly valued in the commercial market, was initiated using seeds as explants. The longitudinal explants from seedlings germinated *in vitro* were cultivated on Murashige and Skoog medium containing 8.8 μ*M* benzyladenine (BA) or 4.6 μ*M* kinetin at pH 7.0. After 120 d, each explant gave rise to five shoots and this number of shoots increased 20–25% after subculture. The hyperhydricity was similar in both media, but callus formation was lower on the medium with BA. The shoot development, in terms of epicotyl length, and fresh and dry weight after 6 wk, was also recorded. The epicotyl length was similar on shoot-forming media but the quality of shoots was better on media containing BA. In about 1 yr, 500-600 well-defined shoots were obtained. The rooting of shoots was very slow and a vigorous radical system was observed after 1 yr of culture.

Key words: cactus; *in vitro* culture; *Pelecyphora*.

Introduction

Mexico has the richest diversity of cacti, with more genera and species than any country in the world. Unfortunately, about 217 species are considered to be endangered plants (Glass, 1997; Norma Oficial Mexicana, 2001). This situation is due mainly to illegal collection and trade, and the destruction of natural habitat. This is particularly true for the genus *Pelecyphora* (McCarthy, 1987). *Pelecyphora aselliformis* was not considered rare until recently because it has been exported at an alarming rate for its high value as an ornamental plant. The type locality has been severely impacted by illegal collection, but there are some relatively undisturbed sites due to the inaccessibility of the area (Sotomayor-Martín del Campo et al., 2000). *P. aselliformis* frequently forms many-stemmed clumps in which several heads arise from the same root system. For cultivation, these separate heads may be cut off, rooted, and propagated as individual plants. However, it is not likely that such vegetative reproduction can occur under natural conditions (Anderson et al., 1994).

Cacti are usually propagated by seeds and rooted offshoots. However, these conventional methods are inadequate for species with seed dormancy, low germination rates, self-sterility, slow growth rates, or few offshoots. *In vitro* tissue culture is an alternative procedure for cactus proliferation since it allows the production of many individuals from a single explant and faster growth rates. Micropropagation systems have been developed for more than 35 species of cacti, representing 20 genera (Starling and Dodds, 1983; Hubstenberger et al., 1989; Clayton et al., 1990; Fay and Gratton, 1992; Pérez-Molphe-Balch, 1998).

P. aselliformis has been included in Appendix I of the Convention on International Trade in Endangered Species (CITES), but recent studies have recommended that it should be transferred to Appendix II (Sotomayor-Martín del Campo et al., 2000). However, this species grows slowly and to reach 1 cm high requires 2–3 yr of growth under greenhouse conditions and more than 4 yr in the wild. Therefore, in this study, we report a micropropagation system through shoot organogenesis. The system allows the production of clonal plants in a shorter time and reduces the need to have field-collected specimens.

Materials and Methods

The seeds of *P. aselliformis* were obtained from the Institute of Research on Desert Zones, from the University of San Luis Potosí. The seeds were surface-sterilized as previously described (Elías-Rocha et al., 1999) and germinated in baby food jars containing half-strength (½) solid MS medium (Murashige and Skoog, 1962). The medium was supplemented with 116 μ*M* *myo*-inositol, 1.2 μ*M* thiamine-HCl, 30 g l^{-1} sucrose, and 3.5 g l^{-1} Phytagel. Cultures were maintained under conditions of 16 h light/8 h dark at 25°C.

To induce shoot formation, the lateral segments of the epicotyls were transferred onto MS medium containing 4.6 μ*M* kinetin (K4) or 8.8 μ*M* benzyladenine (BA) supplemented with 1% activated charcoal (AC). Because the sterilization process in the presence of AC could lower the medium pH by 1 or 2 units (Pan and Van Staden, 1999), the medium pH in this study was adjusted to 9 to obtain a final pH of 7. This pH was selected because *P. aselliformis* grows in soils with pH of 6.8–7. The medium was autoclaved at 120°C and 1 kg cm^2 for 20 min.

Each treatment consisted of three replications, each with five jars with each jar containing three explants. The number of shoots, callus formation, and hyperhydricity (increase of water in tissues) were evaluated after 60, 90,

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and 120 d of culture. Callus formation and hyperhydricity were measured as: 0 (absence), + (low), ++ (moderate), and +++ (high). Shoot growth was evaluated after 30 d in terms of epicotyl length, fresh weight, and dry weight. Dry weight was obtained by keeping the tissues at 90°C for 5 h. Six shoots were evaluated weekly during 6 wk of culture. Data were transformed to $(n + 1)$ to reduce subjectivity and were analyzed by a completely randomized design (Steel and Torrie, 1980).

To induce root formation, individual shoots were transferred to MS and ½MS with 1% polyethylenglycol (PEG) and 1% AC, designated as ½MS-1P. Each treatment consisted of 25 shoots. Root formation was measured after 3, 6, and 12 mo. of culture based on root length: + (≤1 cm), ++ (1–3 cm), and +++ (≥3 cm). Data were also transformed to $(n + 1)$ for statistical analysis. Data from all the experiments described above were analyzed by ANOVA using the Tukey test ($P < 0.05$).

Results and Discussion

The sterilization process of seeds was highly effective but only 10 (55%) out of 18 *P. aselliformis* seeds germinated on the medium. To our knowledge, there is no information about the percentage of seed germination under natural conditions but the *in vitro* germination was low compared with those of other cacti propagated *in vitro* (Fay and Gratton, 1992; Elías-Rocha et al., 1999). As the seeds of *P. aselliformis* did not possess a hard cover, the sterilization process could have damaged the embryo. Another possibility is that some seeds were sterile or dormant. The use of areoles and segments of mature plants was also attempted without success because of high rates of contamination (100%) and tissue oxidation (80%).

TABLE 1

INDUCTION OF SHOOTS FROM *PELECYPHORA ASELLIFORMIS*

Medium	Time (d)	No. of shoots	Hyperhydricity[z]	Callus[z]
B8	60	3.26 a	2.68 a	2.68 a
K4	60	3.02 a	2.88 a	2.88 a
B8	90	3.98 a	2.57 a	2.33 b
K4	90	3.77 a	2.73 a	2.75 a
B8	120	4.97 b	2.59 a	2.59 b
K4	120	4.93 b	2.50 a	2.99 a

Means with different *letters* in the same *column* differed significantly ($P < 0.05$).

[z] Data represent the means of crosses plus one $(n + 1)$ per experimental unit.

Induction of shoots. It has been reported that BA and kinetin were effective for induction of shoots from the cacti *Mammillaria candida* (Elías-Rocha et al., 1999), *Astrophytum myriostigma* (Santos-Díaz et al., 2000), and *Ferocactus latispinus* (Santos-Díaz, 1996). Therefore, these cytokinins were used for shoot induction in *P. aselliformis*. Because BA at 20 μ*M* could induce hyperhydricity of several *in vitro* cultures of apple (Paques and Boxus, 1987), melon, and carnation (Leshem et al., 1988), a lower concentration of BA (8 μ*M*) was used in this study. AC was also added to the medium to reduce callus formation. The beneficial effects of AC are attributed to the absorption of inhibitory substances present in the medium or explant (Weatherhead et al., 1978). Based on these considerations, MS medium was supplemented with 8 μ*M* BA (B8) or 4 μ*M* kinetin (K4) and 1% AC. The results are presented in Table 1. The hyperhydricity and the number of shoots of *P. aselliformis* grown on B8 and K4 media were similar, but callus formation at 90 and 120 d was statistically lower in B8 medium. Since the presence of callus was lower in B8 medium, the shoots were propagated on this medium. In both treatments, about five shoots per explant were regenerated at 120 d. Higher proliferation rates (ranged between 2.15 and 17.5) have also been reported for other Mexican cacti (Pérez Molphe-Balch et al., 1998) and also in our laboratory for *M. candida* (Elías-Rocha et al., 1999) and *A. myriostigma* (Santos-Díaz et al., 2000). It has been found that the rate of proliferation and response and the number of buds in cacti differed among the genera and species studied. Although the

Fig. 1. Shoots from *Pelecyphora aselliformis* cultivated on B8 medium for 120 d.

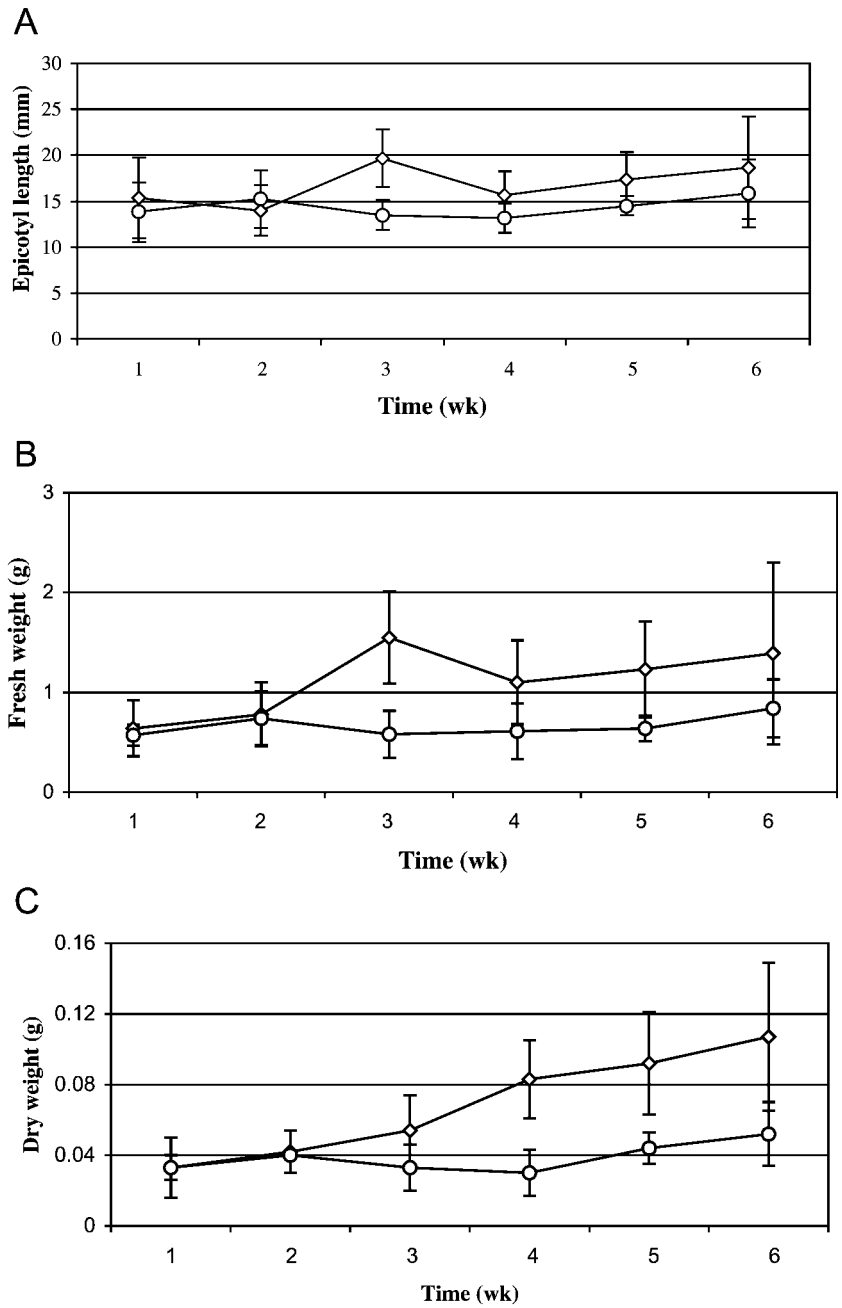

FIG. 2. Development of shoots from *Pelecyphora aselliformis*. The shoots were maintained on B8 (◇) and K4 (○) media. Six shoots were evaluated weekly during 6 wk of culture and data were analyzed by ANOVA.

number of regenerated shoots was low, shoot number could be increased by 20–25% after subculture and, therefore, it was possible to propagate the material rapidly, as illustrated in Fig. 1.

Growth of shoots. To evaluate the growth rate on shoot-forming media, epicotyl length, dry weight, and fresh weight of shoots were measured. In general, an overall increase in epicotyl length for B8 and K4 media was similar but, after 3 wk, there was a two-fold increase in dry and fresh weight in B8 medium compared to K4 medium (Fig. 2*A*–*C*). These results indicate that B8 medium may be better for shoot development since shoots accumulated more dry mass. We also observed that the growth rate of shoots on media B8

TABLE 2

EFFECT OF PEG ON *PELECYPHORA ASELLIFORMIS* ROOTING

Medium	Time (mo.)	Root[z]	Callus[z]
MS	3	1.04 a	2.17 a
½MS-1P	3	1.20 a	1.75 a
½MS-1P	6	1.36 a	1.26 b
½MS-1P	12	3.04 b	1.04 c

Means with different *letters* in the same *column* differed significantly ($P < 0.05$).

[z] Data represent the means of crosses plus one ($n + 1$) per experimental unit.

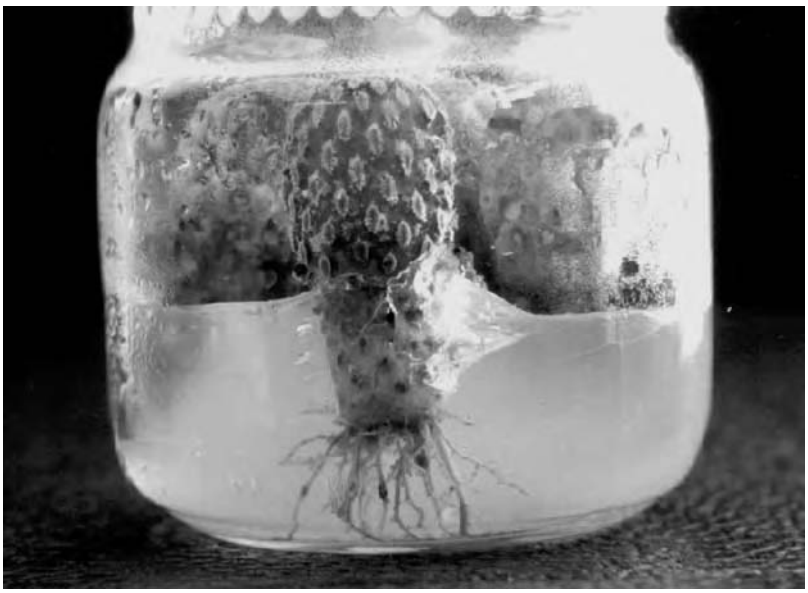

FIG. 3. Rooting of shoots from *Pelecyphora aselliformis* on media with 1% PEG after 12 mo.

was about eight times higher than that of *P. aselliformis* seedlings maintained on MS medium (data not shown).

After a year, the 10 seeds that germinated produced around 600 shoots, whose length ranged between 4 and 45 mm. Because this species requires a minimum of 5 yr to flower, several years would be necessary to obtain this quantity of shoots in its natural environment.

Rooting induction. To promote rooting in cactus, MS without growth regulators has usually been used (Fay and Gratton, 1992). However, in this study, a similar medium induced callus formation on *P. aselliformis* shoots. To reduce callus formation, 1% AC was added to the rooting medium. Because low percentages of rooting and poor survival during acclimatization are due to the hyperhydric status of cultured shoots, PEG was also added to the medium as it decreases the water potential of nutrient solutions without being absorbed and shows no evidence of toxicity (Lawlor, 1970). The use of PEG has been shown to increase the survival rate of micropropagated grape plants (Dami and Hughes, 1997). We found that PEG did not promote rooting in *P. aselliformis* at 3 and 6 mo. (Table 2), but the shoots were dark green in color and callus formation was negligible at 6 mo. Once the compact shoots were obtained, AC was no longer required. The shoots became compact and root formation took place thereafter. A vigorous radical system was observed until 10–12 mo. (Fig. 3). It is not surprising that *in vitro* rooting is a long-term process as the *P. aselliformis* roots represent about 80% of total plant length. Since the rooting was the limiting step during micropropagation, additional strategies should be used to improve root formation. The role of auxins such as indole-3-acetic acid and indolebutyric acid in rooting should be investigated in future studies. However, using the present protocol, it is possible to produce plantlets as high as 3–4 cm after 2 yr.

In conclusion, the procedure developed in this work allowed micropropagation of shoots and plants of *P. aselliformis* in a shorter time than in natural conditions. About 10 shoots were regenerated by each germinated seed and, additionally, the shoot formation rate increased 25% after subculture, improving the yield considerably. It is expected that this biotechnological approach will be an alternative to reproduce *P. aselliformis* on a commercial level in order to satisfy the market demands and thus reduce the need to have field-collected specimens.

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